Structure-guided tuning of a selectivity switch towards ribonucleosides in Trypanosoma brucei purine nucleoside 2' deoxyribosyltransferase

Jon Del Arco, Alberto Mills , Federico Gago, Jesús Fernández-Lucas

Abstract

The use of nucleoside 2'-deoxyribosyl-transferases (NDTs) as biocatalysts for the industrial synthesis of nucleoside analogues is often hindered by their strict preference for 2'-deoxyribonucleosides. We now show that a highly versatile purine nucleoside 2'-deoxyribosyltransferase from Trypanosoma brucei (TbPDT) can also accept ribonucleosides as substrates, most likely because of the distinct role played by Asn53 at a position that is usually occupied by Asp in other NDTs. Moreover, this unusual activity was improved ~3-fold by introducing a single amino acid replacement at position 5 following a structure-guided approach. Biophysical and biochemical characterization revealed that the TbPDTY5F variant is a homodimer that displays maximum activity at 50 oC and pH 6.5 and shows a remarkably high melting temperature of 69 oC. Substrate specificity studies demonstrated that 6-oxopurine ribonucleosides are the best donors (inosine > guanosine >> adenosine) whereas no significant preferences exist between 6-aminopurines and 6-oxopurines as base acceptors. In contrast, no transferase activity could be detected on xanthine and 7-deaza purines. TbPDTY5F was successfully employed in the synthesis of a wide range of modified ribonucleosides containing different purine analogues.